ABSTRACT

The human colon cancer cell line HT29 is morphologically undifferentiated in standard culture conditions. The cells were incubated for 30 s in polyethylene glycol (27%, v/v), then washed, and refed with standard medium. In these conditions of treatment, polyethylene glycol was unable to induce a significant cell multinucleation. Three wk after the treatment, circular “flat-foci” developed in the culture, which consisted of circular monolayers of polarized cells. These subpopulations were isolated, then grown as independent lines (lines 27, 28, 30, and 31) in standard culture conditions, and characterized. Two types of differentiated cells were present in these lines, namely, enterocytic cells and mucus-secreting goblet cells. These characteristics of intestinal differentiation were found to be stable during the long-term culture of these lines in standard medium. We were able to isolate from line 27 a clonal derivative (C1.27H) exhibiting 2 lineages of differentiation, as assessed by electron microscopy, immunofluorescence, and immunoblot analysis of cell membranes with anti-sucrase-isomaltase antibodies, and enzyme activities. Sucrase-isomaltase was present in two forms, namely, the high-molecular-weight precursor and the cleaved subunits. Finally, the C1.27H cells were found to be significantly less tumorigenic than the parental HT29 cells in both in vitro and in vivo tumorigenicity tests. This stably differentiated cell clone could represent the cancer derivative of the normal stem cells of the intestinal crypt. It is therefore a possible model system for the study of intestinal cell differentiation.

INTRODUCTION

A maturational arrest that maintains the neoplastic cells at a certain stage of development has been noted in a variety of malignancies. One of the greatest challenges of cell biology is to understand the origin of this block of differentiation. Several lines of investigation have shown that a breakdown in junctional communication between cells is correlated with the emergence of the neoplastic phenotype (1) and the maturational arrest in epithelial cells in culture (2). These findings have prompted some investigators to test whether it could be possible to restore phenotypic characteristics of differentiation in cancer cells by overcoming the disruption of junctional communications caused by transformation. Interestingly, Steinberg and Defendi have shown that PEG1 treatment of SV-40-infected keratinocytes induced extensive cytoplasmic communications by cell fusion (3). A burst of differentiation paralleled the multinucleation. These findings suggested that cellular interactions produced by PEG fusion might be effective inducers of differentiation (3).

These results prompted us to test whether PEG could be also an effective inducer of differentiation in epithelial cancer cells of glandular origin. We have chosen the human colonic adenocarcinoma cell line HT29 (4) as a model system for this study for several reasons. (a) The HT29 cells are morphologically undifferentiated in standard culture conditions (5-7). When examined with the electron microscope, these cells are found to be unpolarized, and they do not display the specific characteristics of enterocytic cells or of mucus-secreting goblet cells. (b) These cells, however, have the potential to differentiate. A dramatic and partially reversible increase in alkaline phosphatase activity has been observed upon addition of sodium butyrate to the culture medium (8). It has been shown also that changes in the nutritional status of HT29 cells were able to reversibly induce their enterocytic differentiation (5, 7). In contrast to these reversible effects on HT29 cells, it has been shown that cell populations with stable morphological and functional characteristics of intestinal differentiation emerged in culture, following a long-term treatment of these cells with sodium butyrate (6). (c) It is easy with this model to monitor the process of morphological differentiation. The HT29 cells undergoing differentiation display a very particular pattern of growth, referred to as “flat-foci” (6), and they can be easily distinguished from their undifferentiated counterparts with the inverted microscope. Therefore, microscopic examination of the cultured cells is a simple method to perform a first screening of putative differentiation inducers on HT29 cells.

We addressed the issue of a differentiating effect of PEG on HT29 cells using flat-foci as an indication of the emergence of the differentiated populations. We now report that PEG elicited the emergence of typical flat-foci in cultured HT29 cells. In addition, the clonal analysis of the differentiation properties of these flat-foci-forming cells showed that they have the ability to differentiate into both enterocytic and mucus-secreting cells.

MATERIALS AND METHODS

Culture of HT29 Cells. The human colon adenocarcinoma cell line HT29 was obtained from Dr. Fogh (4). The HT29 cells were used between passage 151 and passage 163. They were routinely cultured in DME (Grand Island Biological Co., Grand Island, NY):10% heat-inactivated FBS (Gibco). This medium was designated SM. In routine culture conditions, the culture medium was changed 72 h after seeding and then daily. The cells reached confluency after 7 to 10 days of culture. The cells were transferred weekly using 0.25% trypsin:0.02% EDTA. The cells were periodically screened for Mycoplasma contamination using the method of Chen (9) and were negative for infection.

PEG Treatment. In a first series of experiments, HT29 cells were seeded in 25-cm2 Falcon flasks (Falcon, Oxnard, CA) in standard medium. Four days after seeding, when the cells were in their exponential phase of growth, they were treated with PEG basically according to the experimental protocol of Steinberg and Defendi. Briefly, the cells were incubated in 5 ml of serum-free culture medium containing 27% PEG (v/v) (M, 1000 PEG; Sigma Chemical Co., St Louis, MO) for 30 s and then rinsed in decreasing concentrations of PEG, 16.6% and 7.4% (v/v), respectively. After 4 successive washes in DME, the cells were returned to standard medium and allowed to proliferate to confluency. During the plateau phase of the culture, the medium was changed every day, and the cells were inspected periodically with the phase-contrast microscope. Postconfluent cells were subcultivated using...
0.25% trypsin:0.02% EDTA and were continuously grown in standard medium.

Cell and Nuclei Counts. For cell counts, HT29 cells were dissociated with 0.25% trypsin:0.02% EDTA. The differentiated cell lines were harvested using a 2-step dissociation method, since our preliminary studies had shown that a simple trypsinization was unable to dissociate the cells. Briefly, the monolayers were rinsed with phosphate-buffered saline (in g/liter: NaCl, 8; KCl, 0.2; Na,HPO₄, 1.15; KH₂PO₄, 0.2, pH 7.2) and then they were incubated for 5 min at 37°C in 1 mM EDTA. Then the EDTA solution was discarded and replaced by a solution of 0.25% trypsin:0.02% EDTA. The cells were counted with a hemocytometer. The percentage of multinucleated cells was determined as follows. The cells were centrifuged onto slides, they were fixed in methanol:acetic acid (3:1, v/v), and stained with hematoxylin:eosin. The percentage of multinucleated cells denotes the ratio of cells containing more than one nucleus per cell to the total number of cells × 100.

Flat-Foci. Flat-foci were defined as sharply delimited circular foci of cells organized in a monolayer. These flat-foci were easily identified by examining the confluent cultures with the inverted microscope or with the naked eye.

Isolation of Differentiated Subpopulations and of Clonal Cell Lines. Three mo after PEG treatment, confluent cell cultures containing numerous flat-foci were dissociated with trypsin (0.25%):EDTA (1:5000). Based on our observation that the flat-foci-forming cells were more resistant than the others to the dissociating effect of trypsin, we performed 2 successive trypsinizations to enrich the cell suspension in flat-foci-forming cells. The first harvest was discarded, and a second round of trypsinization was performed. The second harvest was resuspended in standard medium and counted, and a monodispersed cell suspension was seeded into 100-mm-diameter Petri dishes at 200 cells/dish in a culture medium made of Ham's F-12/DME (1/1, v/v)/10% FBS. After 20 to 30 days of incubation, flat colonies were identified with the inverted microscope, and they were isolated using plastic rings and trypsin (6). The flat-colonies were then transferred independently into 35-mm-diameter Petri dishes and grown in SM. They were subsequently transferred to 25-cm² Falcon flasks. These subpopulations were grown as independent cell lines. One of these lines (line 27) was subsequently cloned using a dilution plating technique. Briefly a monodispersed cell suspension was distributed to microtest plates (96-well microtest plates; Falcon, Becton Dickinson, Oxnard, CA) at a mean ratio of 1.5 cells per well. Those wells containing only one cell, as ascertainment by microscopic inspection by 2 independent observers, were identified with their coordinates on the plates. Cells grown in wells observed to initially contain one cell were subsequently transferred to increasingly larger culture vessels. Each clone was designated by a letter following the number of the parent subpopulation (C1.27H, for example).

All subpopulations and clonal cell lines were cultured in standard medium. The cells were routinely subcultured using the above described two-step dissociation method. The cells were periodically screened for Mycoplasma contamination using the method of Chen (9) and were examined the confluent cultures with the inverted microscope or with the naked eye.

Quantitation of Mucus Secretion in Cell Monolayers. Postconfluent monolayers were dissociated, and then they were centrifuged onto slides and fixed with methanol:acetic acid (3:1, v/v), and stained with hematoxylin:eosin. The percentage of multinucleated cells denotes the ratio of cells containing more than one nucleus per cell to the total number of cells × 100.

Transmission Electron Microscopy. For electron microscopy, the cultured cells were fixed in 3% glutaraldehyde and embedded in situ in Epon according to the method of Brinkley and Chang (12). Semithin sections (2 µm) and thin sections (600 Å) were cut perpendicular to the plane of culture with an LKB ultratome (LKB, Bromma, Sweden). Semithin sections were stained with toluidine blue, and thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM109 electron microscope.

Enzyme Assays and Protein Determination. The cells were washed once with phosphate-buffered saline. They were dissociated using the above described 2-step dissociation method (incubation in 1 mM EDTA, followed by trypsinization), and then the cells were centrifuged at low speed and the supernatant was discarded. The cells were then washed 3 times in phosphate-buffered saline by low-speed centrifugation. The pellet was weighed and stored at ~80°C. A homogenate was made in 50 mM mannitol-2 mM Tris (pH 7.1) at 4°C, using a Waring blender (90s-velocity 1), and the homogenate was filtrated through gauze. The examination of the homogenate with an inverted microscope showed that all cells were lysed by this procedure. Subcellular fractionation was performed by the method of Schmitz (13). This method is based on the sequential use of CaCl₂, differential centrifugation, and Tris (13). The activities of disaccharidases were measured in both the homogenate and the fraction enriched in "brush-border membranes" (P2 fraction), as previously described (14). Proteins were estimated according to the method of Lowry et al. (15) with crystalline bovine serum albumin as a standard. The enzyme activities are expressed as milliunits per mg of brush-border proteins. One unit is defined as the activity that hydrolyzes 1 µmol of substrate per min under the experimental conditions.

Anti-Saccharose-Isomaltase Antibodies. Polyclonal monospecific anti-human SI antibodies were obtained by injecting pure human SI of human Blood Group O into rabbits, and IgG was prepared as previously described (16).

Immunofluorescence. Indirect immunofluorescence was performed in situ on cells grown in 25-cm² flasks, as described by Zweibaum et al. (7). Briefly, the cultured cells were rinsed with phosphate-buffered saline and fixed for 20 min at room temperature in 3.7% paraformaldehyde (prepared in phosphate-buffered saline). Then they were incubated for 30 min with anti-human small intestinal SI IgG (0.1 mg/ml) prepared in the rabbit. After 3 washings in phosphate-buffered saline, they were incubated under the same conditions with fluorescein-conjugated sheep anti-rabbit immunoglobulin (IgG) (Institut Pasteur, France), diluted 1:100, and washed again 3 times. The cells were then examined with a microscope equipped with epifluorescence (Nikon) and photographed.

Polycrylamide Gel Electrophoresis, Western Blot, and Immunological Detection. Membrane proteins of the P2 fraction were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, according to the method of Laemmli (17) on 7.5% polyacrylamide gels. The separated proteins were transferred to nitrocellulose sheets (Western
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RESULTS

Characteristics of HT29 Cells Growing in Standard Medium. When cultured in SM, HT29 cells grow as multilayers. Electron microscopy showed that the cells were unpolarized and morphologically undifferentiated (Fig. 1). The cells were joined by numerous desmosomes. Tight junctions were not found, in keeping with our own observations (6) and those of others (20). Microvilli were occasionally observed on the cell membranes, but typical brush-borders were always absent.

To examine whether some mucus-secreting goblet cells were present in the HT29 line, we determined the MI on post-confluent cultures. As shown in Table 1, the number of mucus-secreting cells was very low. The immunofluorescence reactions performed on confluent cultures of HT29 cells with anti-SI antibodies showed that the surface of the cell layer was entirely negative.

The absence of labeling of HT29 cells with anti-SI in the immunofluorescence assay was in keeping with the enzyme assays showing the absence of any significant sucrase activity (Fig. 2) and also with electron microscopy showing the absence of any enterocytic differentiation in HT29 cells (Fig. 1).

Effects of PEG on HT29 Cells. The treatment of the HT29 cells with 27% PEG (v/v) for 30 s did not elicit any significant change in the percentage of the multinucleated cells in the treated population versus control. The percentage of multinucleated cells did not exceed 0.4% of the total number of cells.

Fig. 3 shows that 48 h after the treatment, the cell densities were significantly lower in the treated cells than in the control HT29 cells. Three wk after PEG treatment, circular flat-foci developed in the culture. They were readily identified with the phase-contrast microscope, contrasting with the unmodified surrounding cells (Fig. 4). Postconfluent cells were passaged and were continuously grown in SM. These flat-foci developed regularly at the plateau phase of growth during the successive subcultures in SM. In routine culture conditions, the cells were passaged every 20 days. Since some of these flat-foci stained metachromatically with toluidine blue, it was suggested that they represented differentiated, mucus-secreting cell populations. This was confirmed by electron microscopy, which disclosed 2 types of differentiated cells in the postconfluent cultures: mucus-secreting goblet cells and enterocytic cells with a typical brush-border at their apical surface. All these differentiated cells were polarized. Their apical membranes were separated from the basolateral membranes by typical junctional complexes. In addition, immunofluorescence reactions with anti-SI antibodies disclosed strong reactivities of the apical membrane of isolated cells and of some cell clusters.

Table 1 Fraction of mucus-secreting and of enterocytic cells in 4 cell lines and in the parental HT29 cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>MI (%)*</th>
<th>% of sucrase-isomaltase-positive cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line 27</td>
<td>49.27 ± 0.11</td>
<td>20–30</td>
</tr>
<tr>
<td>Line 28</td>
<td>19.36 ± 0.41</td>
<td>20–30</td>
</tr>
<tr>
<td>Line 30</td>
<td>24.95 ± 1.96</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Line 31</td>
<td>33.54 ± 1.44</td>
<td>&lt;10</td>
</tr>
<tr>
<td>HT29 (control)</td>
<td>0.05 ± 0.01</td>
<td>0</td>
</tr>
</tbody>
</table>

* See "Materials and Methods" for MI equation. Each value represents the mean ± SEM of three independent experiments with three determinations per experiment.
* Semiquantitative determinations performed on 2 flasks for each cell line.

Fig. 1. Low-magnification electron micrograph of a vertical section of post-confluent HT29 cells, showing multilayer of unpolarized and undifferentiated cells. × 1650.

Fig. 2. Enzyme activities of sucrase and maltase measured in parent cell line (HT29) and in PEG-treated clone (CL27H). H, homogenate; P2, fraction enriched in brush-border membranes.

Fig. 3. Growth curves of control and treated HT29 cells during the 4 days following PEG treatment. HT29 cells were seeded in SM at 500,000 cells/25-cm² flask. The cells were incubated for 30 s in PEG at Day 6. Growth was measured at various time intervals following exposure of the cells to PEG. Points, mean of 3 flasks; bars, SEM.
PEG RESTORATION OF HUMAN COLON CANCER CELL DIFFERENTIATION

Experimental Conditions Influencing the Emergence of Flat-Foci. The above described experiments showed that PEG was able to induce the emergence of differentiated populations in HT29 cells. The effects of varying conditions of PEG treatment were also examined. Table 2 summarizes the different experimental conditions of treatment. When we increased the PEG concentration, as well as the duration of the treatment, we observed the occurrence of a burst of multinucleation immediately after the treatment. Then the multinucleated cells disappeared from the cultures, and growth resumed as mononuclear cells. Increased concentrations of PEG had no major effect on the timing of differentiation. We also found that a low PEG concentration, which was otherwise unable to induce any detectable multinucleation, had the capacity to induce the emergence of flat-foci. However, it is impossible to rule out the possibility that some flat-foci originated from very rare fused cells.

Isolation and Characterization of Differentiated Subpopulations. PEG-treated cells enriched in flat-foci-forming cells were dissociated and seeded at clonal density (200 cells/100-mm-diameter Petri dish). After 3 to 5 wk of growth, flat colonies were readily recognized under the inverted microscope, and they were isolated with plastic rings and then grown as independent cell lines.

Among the 7 lines isolated so far, 4 (lines 27, 28, 30, 31) were characterized. All these subpopulations formed at their plateau phase of growth polarized sheets of cells, whose apical surfaces were joined to the basolateral membranes by typical junctional complexes. Mucus-secreting cells were present in all subpopulations. These cells had the ultrastructural characteristics of the goblet cells of the intestine. As shown in Table 1, the percentage of mucus-secreting cells, as determined by a metachromatic staining of cytocentrifuge preparations, was high in line 27 and in line 31. The immunofluorescence staining of the confluent cultures with anti-Si disclosed strong reactivities in lines 27 and 28 (Table 1). The staining pattern was highly characteristic. Specific membrane labeling was detected as fluorescent dots which were restricted to the apical cell surface. The labeled cells were either isolated or grouped into clusters. In contrast, lines 30 and 31 contained very few positive cells.

To further examine the differentiation properties of the progenitors of both the enterocytic and mucus-secreting cells, we performed a clonal analysis of line 27 using a dilution technique. Ten clones were isolated. Table 3 shows that 7 of the 10 clones expressed the 2 types of differentiation. C1.27H is a good example of this pattern of differentiation. The apical surface of C1.27H was covered with a mucus gel which was produced by typical goblet cells (Fig. 5). In addition, electron microscopy disclosed numerous enterocytic cells, endowed with a typical brush-border (Fig. 6). Immunoreactive Si was present in this clone as shown by immunofluorescence staining of the cells in situ (Fig. 7). Immunoblot analysis of the brush-border membrane proteins with anti-Si showed 2 major bands with apparent molecular weights of 260,000 and 140,000, respectively, and a faintly stained band with a molecular weight of approximately 60,000 (Fig. 8). The upper band (M, 260,000) corresponds to...
the uncleaved form of SI, the $M_1$ 140,000 band corresponds to the subunits, and finally, the $M_6$ 60,000 band is a proteolytically degraded form. This profile was very similar to that of a brush-border membrane fraction from a normal adult small intestine which served as a control. This pattern of immunoreactivity was expressed in CI.27H cells at a much higher level than in HT29 cells. Finally, the SI activities were found to be higher in CI.27H than in HT29 cells (Fig. 2). This activity was localized in the CaCl$_2$-precipitable membrane fraction which was assumed to contain the brush-border membrane of the differentiated cells.

It should be pointed out that all these subpopulations and these clonal cell lines retained in long-term culture their characteristics of differentiation. The occurrence of differentiation depended on the phase of growth of the cultured cells. The characteristics of differentiation were absent during the exponential phase of growth, and the differentiation process took place regularly when the cells reached confluency.

The doubling times of 3 cell lines and of HT29 cells were determined on the growth curves during the exponential phase of growth. They were 24.8 ± 0.7 h, 30.9 ± 2.7 h, 25.4 ± 2.5 h, and 26.9 ± 3.3 h for the CI.27H, CI.27J, line 31, and HT29, respectively.

The growth of tumors in nude mice. Athymic male nu/nu mice were inoculated with $2 \times 10^7$ cells. Tumor development was monitored weekly by palpation, and the volume ($V$) calculated as described in "Materials and Methods." Points, mean of 6 different tumors; bars, SEM.

Tumorigenicity Tests. The purpose of these experiments was to examine whether the differentiated populations were less tumorigenic than the parental HT29 cells. In fact, only the CI.27H proved to be significantly less tumorigenic than HT29 cells in both the in vitro (Table 4) and in vivo (Fig. 9) tumorigenicity tests. The other cell lines (CI.27J and line 31) exhibited
only a reduced cloning efficiency in soft agarose.

Microscopically, all the tumors were adenocarcinomas. Well-organized glandular structures with papillary projections were preeminent in Cl.27H tumors (Fig. 10A). Some glandular structures were observed in Cl.27J and in Cl.31 tumors. They were very rare in HT29 tumors (Fig. 10B). Large areas of necrosis were found in all tumors.

DISCUSSION

Previous studies have examined the effects of PEG on undifferentiated cells. Steinberg and Defendi (3) have shown that PEG was able to restore differentiated functions in a system of SV-40-transformed keratinocytes. PEG treatment of these cells induced a burst of terminal differentiation. This transitory effect was concomitant and directly related to the fusogenic effect of PEG. These findings suggested that the differentiation was due to the creation of intercellular coupling by PEG treatment.

In this work we tested whether PEG was able to restore in HT29 cells differentiated functions. Our demonstration of the emergence of typical flat-foci after PEG treatment was a clear indication that the PEG induced the differentiation of HT29 cells.

In contrast to the findings of Steinberg and Defendi, our studies showed (a) that the extent of differentiation was apparently not correlated with the extent of cell multinucleation, (b) that a long delay separated the PEG treatment from the emergence of the flat-foci, and (c) that the flat-foci were stable in standard culture conditions. All these findings suggested strongly that, in our system, the PEG-induced differentiation did involve a mechanism different from that implicated in the experiments of Steinberg and Defendi.

Although we do not know the mechanisms underlying the process of differentiation triggered by the PEG treatment, we suggest that the primary event was a modification of some components of the plasma membrane of the colonic cancer cells by this agent. Since we were unable to find polarized differentiated cells in the parental HT29 cell line, it is unlikely that the PEG has only selected a minor subpopulation in these cells.

Among the stably differentiated lines isolated so far from the HT29 cells after the PEG treatment, the clonal derivative Cl.27H served as a model system to characterize the expression of the enzyme SI. This enzyme is of particular interest as a marker of differentiation of the enterocytic cells for several reasons. (a) Sucrase-isomaltase is one of the best known brush-border enzymes, and this enzyme has been purified to homogeneity from the human intestine (21). It is an intrinsic protein of the intestinal microvillus membrane, which is synthesized as a single high-molecular-weight precursor, then inserted into the apical membrane, and cleaved into 2 components (sucrase and isomaltase) of lower molecular weights (22) by pancreatic proteases. (b) This enzyme is restricted to the brush-border domain of the intestine, in contrast to other brush-border enzymes such as aminopeptidase or alkaline phosphatase, which are ubiquitous (23). (c) Several lines of investigations have shown that this marker of enterocyte differentiation is expressed by some human primary colonie tumors (24), by the Caco-2 (25) and LIM1863(26) cell lines maintained in SM, and by some other human colonie cancer cell lines maintained under specific culture conditions (5, 27, 28).

Hauri et al. (29) have recently shown that SI is synthesized in the cultured Caco-2 cells as a single chain precursor which is not cleaved at the plasma membrane. In contrast to this, we showed by immunoblot analysis that the Cl.27H cells did express both the high-molecular-weight precursor of SI and the cleaved subunits. The undifferentiated HT29 cells displayed the same pattern as Cl.27H. The bands, however, were very faintly stained. This finding was rather surprising, since we did not find any significant enzymatic activity in these cells nor any positivity with anti-SI in the immunofluorescence assay. Taken together, these findings suggest that the SI was synthesized at a low level in the undifferentiated HT29 cells and then abnormally processed. It is noteworthy that SI is transferred toward the brush-border of the enterocytes through intracellular vesicles which can fuse with lysosomal vacuoles where SI can be degraded before its insertion on the membrane (30). It is therefore tempting to suggest that, in the undifferentiated HT29 cells, which are unpolarized and which have no brush-border, the SI is continuously synthesized at a low level and then degraded in lysosomes. This conclusion is in line with the findings of Trugnan et al. (31) who have recently shown that SI cannot be detected by means of cell surface immunofluorescence in HT29 cells. Pulse experiments with [35S]methionine showed that the enzyme is, however, synthetized in these undifferentiated cells and then rapidly degraded (31). Finally, the isolation of a multipotential clonal line (Cl.27H) provided direct evidence that the 2 differentiation lineages, namely, enterocytic cells and mucus-secreting cells, originated from a unique precursor. This finding is in keeping with that of Cox and Pierce (32) who demonstrated an unitarian origin to multiple cell lineages by using clonal derivatives of a transplantable carcinoma of the rat as an in vivo model of cell differentiation.

Our clonal analysis of differentiation also suggests that our
PEG treatment has resulted in the conversion of clones of HT29 cells into stably determined, but undifferentiated stem cells whose progeny had the potential to differentiate into multiple cell lineages when reaching confluency.

It is tempting to speculate that the Cl.27H may represent a cancer derivative of the multipotent stem cell (33) of the normal intestinal crypt. This new clonal cell line may therefore represent a unique model system for studies aimed at elucidating the mechanisms of epithelial cell differentiation within the epithelial crypt.

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Restoration by Polyethylene Glycol of Characteristics of Intestinal Differentiation in Subpopulations of the Human Colonic Adenocarcinoma Cell Line HT29

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